PRELIMINARY INVESTIGATIONS IN THE USAGE OF GAS CHROMATOGRAPHY FOR THE DETECTION OF LIFE ON MARS

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The technique of gas chromatography as applied to the identification of pyrolysis products of biological material has been advanced earlier as a means of life detection on Mars. (Ref. 1, 2) Preliminary studies as submitted here confirm in part the feasibility of this concept.

The premise upon which this concept is based originated from the fact that terrestrial life is an aggregate of carbonaceous macromolecular structures as well as a lesser mass of monomeric organics; the latter arise as intermediates in the syntheses of the macromolecules or by-products of metabolic processes. The proteins, polysaccharides, and nucleic acids which represent macromolecular structure are composed of basic building blocks such as the amino acids, monosaccharides, lipids, purine and pyrimidine bases. These constituent substances are distributed universally amongst living materials, and the essential components of the protoplasm of life require that critical proportions of these basic building blocks be maintained either by assimilation from the environment or manufactured by the organism.

The simple technique of thermal degradation has been applied to the analyses of chemical structure. (The literature is replete with references too numerous to iterate.) However, in a complex organic aggregate such as life, the pyrolyzates from thermal degradation present a relatively difficult task of determining the original contributing structures.

Nevertheless, it is possible to present a chromatographic picture (such as has been described in paper chromatography in the two dimensional display of peptides for the elucidation of protein structure) which could similarly describe the presence of life.

Freshly isolated, but unidentified, microorganisms were grown in trypticase soy broth under aerobic conditions at 30°C. These were harvested during log phase of growth, washed with distilled water several times, and lyophilized to dryness.

A single strain of microorganism was used to determine the optimum temperature of pyrolysis which would give us the major amount of volatile components of thermal breakdown as determined by a hydrogen flame detection device from gas chromatography column separations. Since organics were of particular interest, this type of detector incorporated in the Perkin Elmer Model 800, was used for this purpose. 0.12" x 12' column of 15% SE 30 on 60/80 mesh chromasorb W was maintained isothermally at 200°C. The flow of gas was 80 mls/min.

The pyrolysis chamber was a stainless steel loop attached onto a linear 6 way valve, paired with another linear valve, which permitted, initially, evacuation of the sample loop, filling of the pyrolysis chamber with inert helium atmosphere, pyrolysis under closed system, and sweeping of the pyrolysis chamber into the flowing gas system directed

into the columns. The valving and tubing between the pyrolysis chamber and column was maintained at 125°C during the course of analysis. Mass of the components appearing at the detector was measured by an Infotronics digital readout system. A weighed sample of material (10 to 200 μg) was funneled into the sample loop and a heat pretreated fibre glass cordage was inserted into the sample loop to prevent particulate matter from entering the critical valving area. A pair of heated nickel plates, whose temperature was regulated by a temperature controller and held together by spring action was slipped over the loop making direct thermal conductive contact with the stainless steel sample loop.

Pre-selected periods of time for various temperatures were utilized to determine the efficacy of residence time and temperature to give the greatest number of products. Figure 1. shows that the total integrated products sensed at the detector are a function of these two parameters. No significant products are produced at temperatures of 300°C. Exposure for 4-8 minutes will tend to decrease the total mass of substances. Optimum temperature and residence time was 450° C or 500°C for 2 minutes. It is not indicated, but to be noted that the temperature within the stainless steel tubing took approximately 45 secs. to attain 450°C.

Figure 2, shows that the volatiles produced by thermal degradation tend in turn to be degraded further. Components of thermal degradation

as determined by retention times show peak production at various times, e.g., a component of retention time 56-57 secs. (uppermost curve) is produced maximally at three minutes residence time for the specified temperature of 500°C. The amount of product so produced will decrease as time of residence is increased. It is to be assumed that synthesis of a thermal product is concurrently in progress with its degradation. The accumulated products of thermal decomposition represent only the thermal product which is measurable at the instant that the vapors are swept into the column. The figure also shows that certain products are decomposed rapidly in time, while others reach a maximum at 4 minutes and appear to be stable at these temperatures. Others, on the other hand, reach a maximum yield and degrade relatively slowly. The figure shows the importance of reproducing the exact conditions of pyrolysis so that repeatability in terms of relative peak heights may be obtained.

In order to ascertain as many of the products of thermal decomposition as is possible with a single system, a 0.010" capillary column 300 ft. long coated with diethylene glycol succinate polyester was temperature programmed for the analyses of some microorganisms isolated from soils. Typical results are indicated in Fig. 3A. It can be seen that under these reproducible conditions during linear temperature programming, the chromatographic peaks descriptive of the retention products

occur with a periodicity common to all the organisms. There are differences in peak height relative to adjacent peaks but the chromatograms indicate that thermal decomposition products of microorganisms can be displayed in a fashion to show equivalence. The relative peak heights in each separate chromatogram show differences and may indicate differences in the relative amounts of precursor organic substance.

Figure 3B shows the pyrolyzate chromatogram of crystalline bovine albumin under similar conditions. It is to be noted that similar patterns corresponding to early appearing chromatographic retention times of the microorganisms are reproduced. It is not altogether surprising, considering the original premise, to find similar patterns from proteins whether they be of plant or animal origins.

Further work is in progress to determine what these pyrolyzate products are, the determination of what precursor organic substances are involved, as well as comparative chromatograms of microorganisms as an approach to taxonomic differentiation.

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Oyama, V.I., Lunar and Planetary Exploration Colloquium, III, 29, (1963).

Oyama, V. I., Vango, S. P., and Wilson, E. M., ARS Journal, 354, (March, 1963).

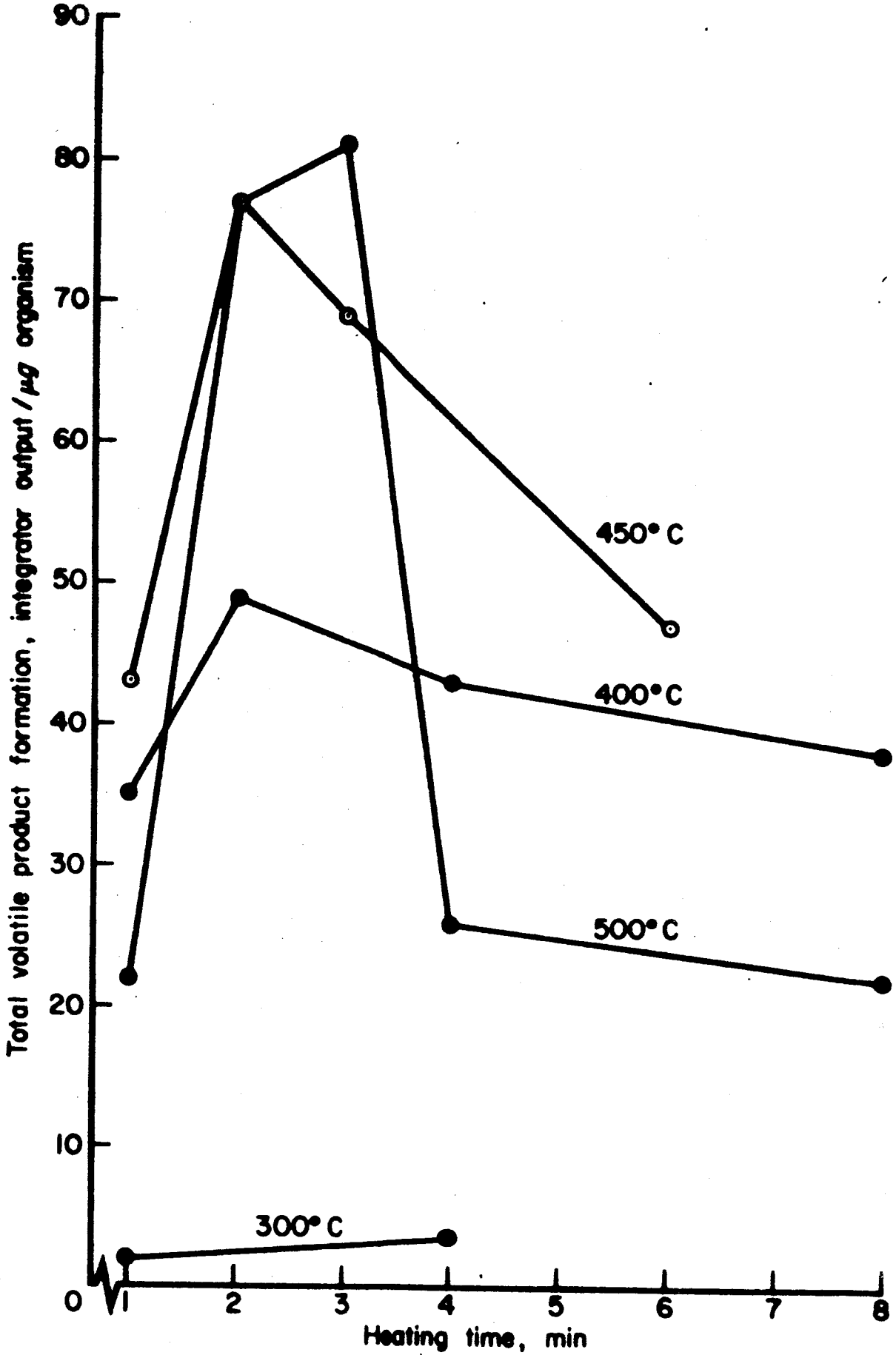

Total volatile product formation, integrator output / μg organism
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
Heating time, min
450° C
400° C
500° C
300° C

Fig. 1

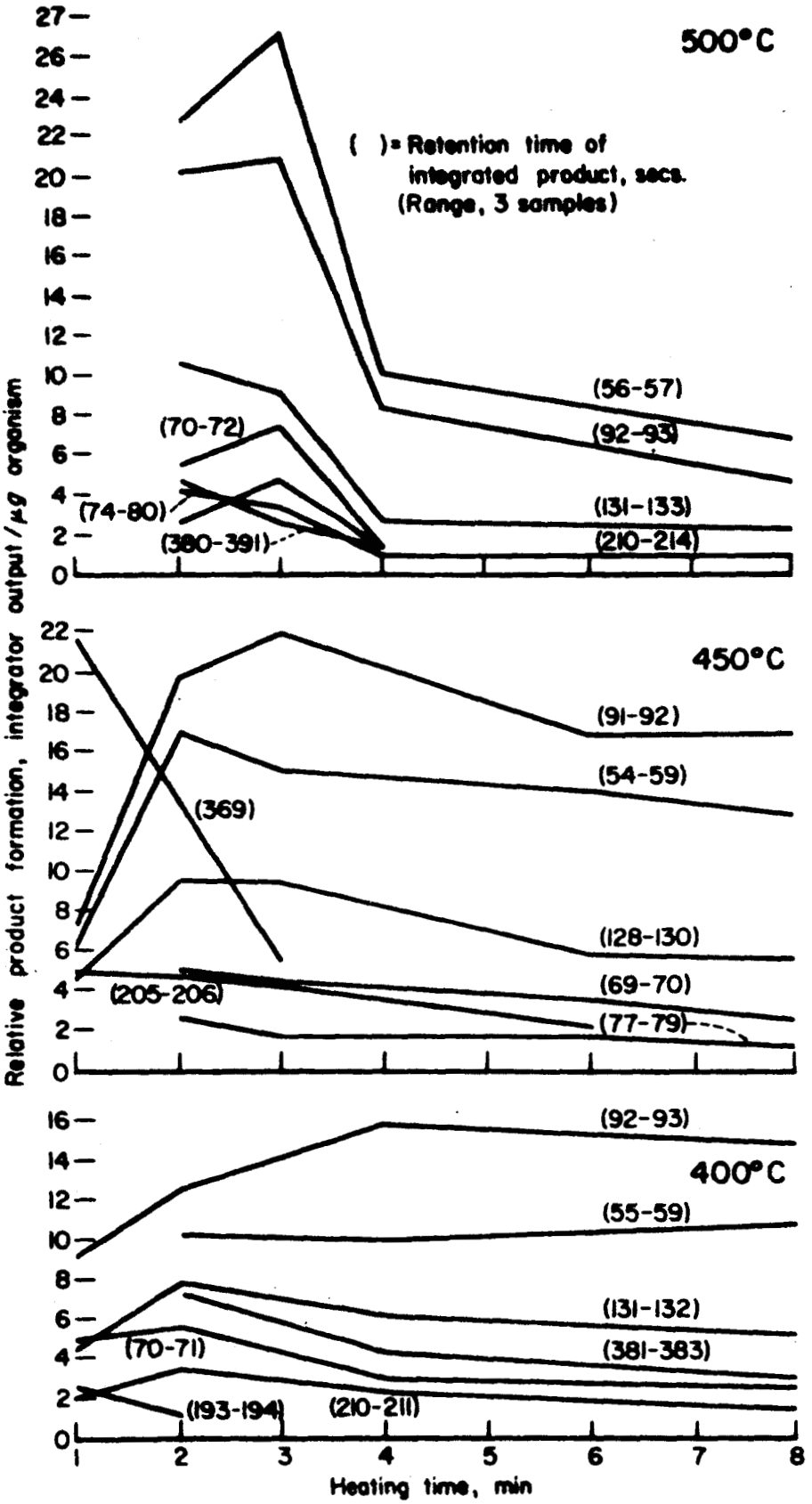
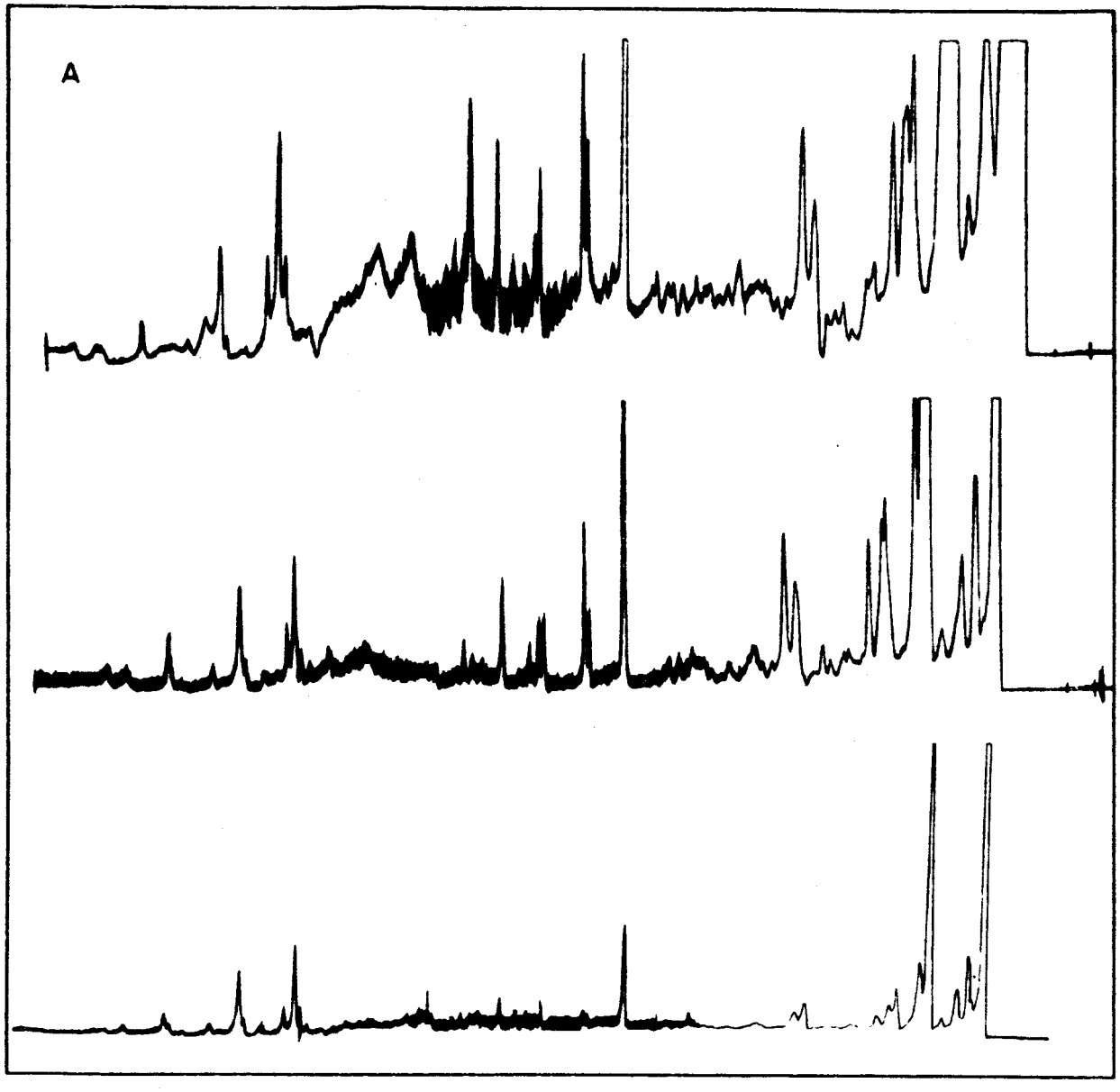

500°C
( ) = Retention time of integrated product, secs. (Range, 3 samples)
(56-57)
(92-93)
(70-72)
(74-80)
(131-133)
(210-214)
(380-391)
450°C
(91-92)
(54-59)
(369)
(128-130)
(205-206)
(69-70)
(77-79)
400°C
(92-93)
(55-59)
(131-132)
(70-71)
(381-383)
(193-194)
(210-211)
Relative product formation, integrator output / µg organism
Heating time, min

Fig. 2



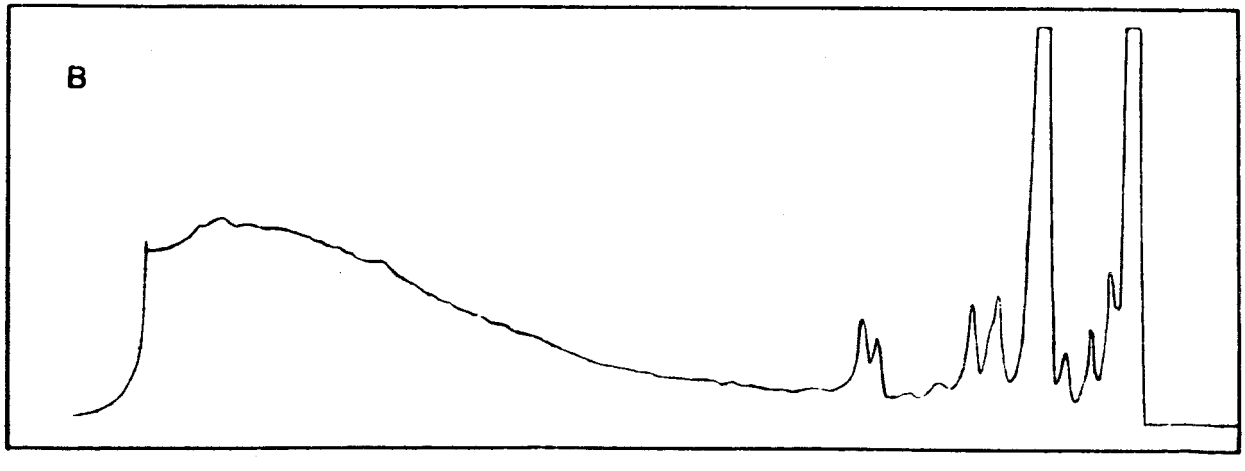


Fig. 3

LIST OF FIGURES